FORM-REGULATION IN CERIANTHUS, VI.

Certain Special Cases of Regulation and their Relation to Internal Pressure.

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In this paper are described certain regulative phenomena of interest both from a descriptive point of view and because they appear to offer further evidence upon the problem of the rôle of internal water-pressure in regulation. At present I can see no other satisfactory interpretation of these cases than that based upon internal pressure. In any case, however, they are interesting modifications of the typical course of regulation and as such deserve mention.

The Oral Regeneration of Oblique Pieces.

Pieces with oblique ends are obtained by sectioning the body at various angles to the principal axes as indicated in Fig. 1. Such pieces show a very marked difference in rapidity of regeneration on the different parts of the oblique cut surface, regeneration being most rapid on that part which represents the region nearest the oral end of the parent body and least rapid on the opposite side. This difference is due in part to the fact discussed in my second paper ('03*b*), viz., that the rapidity of regeneration always decreases with the increasing distance of the regenerating surface from the oral end of the original animal. But the difference in the rapidity of regeneration on the different parts of the oblique surface is considerably greater than that between control pieces with transverse cut surfaces at corresponding levels. It follows from this that some other factor in addition to the difference of level is concerned in the result.

Before proceeding to the description of experiments it should be said that the possible significance of oblique pieces was not fully recognized during the course of my experiments, therefore my study of such pieces was less extended than it would otherwise have been. The data are sufficient, however, to show clearly the consequence of cuts of this kind. *C. solitarius* was used for those experiments.

Series 9.

September 12, 1902.— Three specimens of *C. solitarius* were cut obliquely at two different levels as indicated in Fig. 1; the pieces used for experiment in each case being the portions between the two oblique lines. These pieces possessed an oral and an aboral oblique surface. Unfortunately no control pieces with transverse cut surfaces were made but the records in my notes of a set of pieces which were cut at the same time and kept in the same aquaria will serve as controls. These pieces were prepared as follows: after the removal of the oral end from two specimens by means of a transverse cut just aboral to the œsophagus the remaining portion of the body was cut into three equal pieces (Fig. 2). These pieces may be designated as *A*, *B*, and *C*, *A* being the most oral of the three. By comparison of Figs. 1 and 2 it is seen that the oral end of the control piece *A* is at almost the same level as the most oral portion of the oblique piece, the latter being slightly more oral, while the oral end of piece *B* corresponds very closely in level with the most aboral portion of the cut surface of the oblique pieces. In sectioning living animals so con-

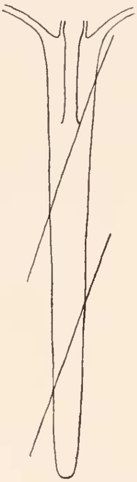

FIG. 1.

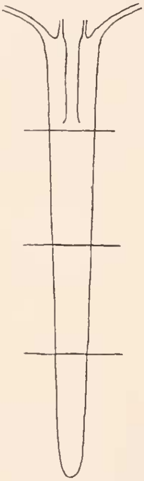

FIG. 2.

tractile and changeable in form as *Cerianthus* the correspondence between the levels of cuts on different specimens is always only approximate. In this case the levels at which the oblique cuts were made differ more or less in the three specimens as well as the levels of the transverse cuts in the controls. Any control is therefore only approximate and the pieces used for this purpose here are as satisfactory in this respect as those employed in other experiments on *Cerianthus.*

In the control pieces *A* and *B* the rapidity of regeneration is that characteristic of the level of the body at which the oral ends of the pieces lie. Comparison of this rapidity with that of the extreme levels of the oblique cut surfaces will show whether the obliquity of the cut has any effect on the rapidity of regeneration.

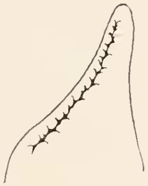

FIG. 3.

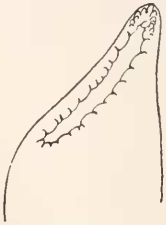

FIG. 4.

After the operation the collapsed oblique pieces acquired a form like Fig. 3. During the next day or two the cut edges rolled inward still more closely and the pointed oral portions became more or less bent over toward the cut surfaces. All became more or less flattened as they lay upon the flat glass bottom of the aquaria.

September 15. — 3 days after section. Experimental pieces: One piece with a few minute tentacle-buds at the uppermost portion of the oblique cut surface; others without tentacles.

Controls. All without tentacles.

September 17. — 5 days after section. Experimental pieces: All the pieces were completely or nearly closed orally by new tissue: the aboral ends were not completely closed by new tissue but were closely approximated and two of the pieces were slightly distended. Each of these pieces possessed a small group of minute marginal tentacle-buds at the extreme oral part of the oblique surface (Fig. 4). No traces of tentacles were visible elsewhere. In the third piece the unpigmented tentacular ridge was visible at the most oral portion of the oblique surface but tentacle-buds had not yet appeared.

Controls. *A.* Closed and distended: marginal tentacle-buds just appearing on the whole circumference; slightly less advanced than the most oral portion of the two experimental pieces with tentacles. *B.* Closed and more or less distended but no tentacle-buds visible.

September 19. — 7 days after section. Experimental pieces: All closed and distended: marginal tentacles on the most oral portion of the oblique surface 2–3 mm.; from this region decreasing in length on each side, the lower three fourths to two thirds of the oblique surface still without tentacles.

Controls. *A.* Marginal tentacles 2–3 mm. about whole circumference. *B.* Marginal tentacles 1–1.5 mm. about whole circumference.

September 22. — 10 days after section. Experimental pieces: Marginal tentacles longest on upper side of oblique disc, decreasing to 0 toward lower side. In none of the three specimens are tentacles present on the lowest portion of the oblique disc: in one specimen the longest tentacles are 5–6 mm. and the circle of tentacles is nearly complete (Fig. 5); in the second the longest tentacles are 3–4 mm. and the lower one third of the disc is without tentacles; in the third the longest tentacles are 2–3 mm. and about one half of the disc is still without tentacles. This piece has been somewhat retarded throughout by irregular inrolling at the aboral end delaying closure and distension.

On the three more advanced pieces labial tentacles are appearing on the upper one third to one half of the disc (Fig. 5).

Controls. *A.* Marginal tentacles 5–6 mm. about whole circumference. *B.* Marginal tentacles 3–4 about whole circumference.

September 26. — 14 days after section. Experimental pieces: In the most advanced piece (Fig. 6) the longest marginal tentacles on the upper side of the disc are 10 mm., the shortest on the lower side 2 mm. Labial tentacles are present on the upper two thirds of the disc, the longest being 3–4 mm. (Fig. 6).

In the second piece the tentacles are equal in length to those of the first and show a similar arrangement.

In the third (delayed) piece the longest marginal tentacles on the upper side of the disc are 8–9 mm. while on the extreme lower portion only minute buds are present. Labial tentacles are present on the upper half of the disc, the longest being 2–3 mm

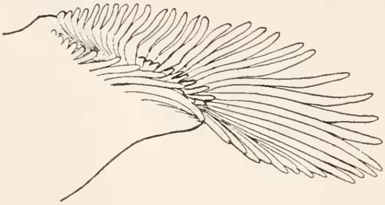

FIG. 5.

In all cases the disc is becoming less oblique (cf. Figs. 5 and 6).

Controls. *A.* Marginal tentacles 8–10 mm. about whole circumference. Labial tentacles somewhat irregular in length 1–4 mm. *B.* Marginal tentacles in one piece 8–9 mm., in other 5–6 mm. Labial tentacles in one piece 1–4 mm., in other about 1 mm.

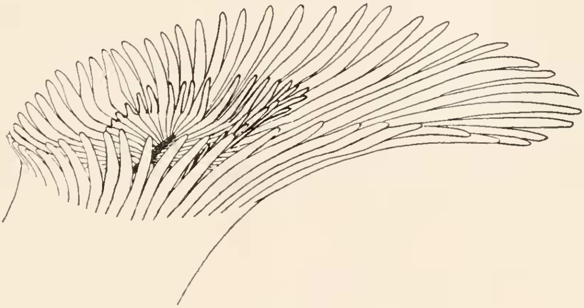

FIG. 6.

Beyond this point the controls need not concern us. The experimental pieces were observed at intervals during three weeks more. During that time there was little increase in length of the longest tentacles, but the shorter tentacles continued to grow until the asymmetry was only slight: in other words the tentacles on the upper side of the disc completed their regeneration earlier than the others. Meanwhile the disc gradually became less and less oblique until it too was almost symmetrical in position. In all pieces the obliquity of the disc was much less when the animal was distended and oriented in the characteristic position with oral end uppermost than when it was strongly contracted. In the later stages the disc did not appear at all oblique except in the contracted condition.

The rates of regeneration in the three experimental pieces were slightly different owing to the delay in closing in some, but if we take the most advanced of these pieces and compare it with the two controls it is seen that there is a marked difference between the lower portion of the disc in the experimental piece and the controls *B*. In the controls *B* the tentacles were 1–1.5 mm. seven days after section and must therefore have appeared on the sixth day, since they were not distinct on the fifth day. In the most advanced experimental piece the tentacles had not appeared on the lowest portion of the disc on the tenth day after section; four days later, however, they were about 2 mm. in length and must therefore have appeared about the twelfth day. There is then a delay of about six days in the appearance of the marginal tentacles on the lowest part of the oblique disk as compared with the time of appearance of the tentacles on a transverse disc at the same level of the body, and in the other experimental pieces the delay is still greater. It is evident then that the retardation in tentacle regeneration on the lower portion of an oblique piece is much greater than the retardation due to differences of level in transversely cut pieces. The data of the experiment and Figs. 5 and 6 show that the appearance of the labial tentacles is similarly delayed.

As regards the time of appearance of the marginal tentacles on the uppermost portion of the oblique disc and on the control pieces *A*, the fact that tentacles appeared in the most advanced experimental piece in three days, while in the controls they appeared somewhat later, indicates that tentacle regeneration is probably slightly accelerated in the upper portion of the oblique discs. According to my notes this is the only case observed in all of my experiments in which tentacles appeared in three days. It is barely possible that the oblique cut in this piece passed so far orally that small stumps of a few tentacles remained. In view of this possibility little stress can be laid on the apparent acceleration in tentacle-regeneration in this one piece, and unfortunately

I have at present no further evidence upon this point, as my attention was not directed to it early enough. I am inclined to believe, however, that there may be a slight acceleration here, provided the inrolling of the margins is not so irregular that closure is delayed, as is frequently the case in oblique pieces.

Leaving this point for future experiments to decide, the retardation of regeneration on the lower portions of the oblique discs is sufficiently evident and requires explanation. In the course of my experiments several other pieces were cut obliquely and always with the same result, viz., appearance of the tentacles first on the uppermost portion of the disc and from this region on each side in succession toward the lowest portion. The tentacles on the lowest portion always appeared later than in transversely cut pieces at the same level.

As in the case of typical regeneration from a transverse cut surface, the presence or absence of a cut aboral end and the plane of the cut if present do not affect the oral regeneration except in so far as irregular or delayed closure of the aboral end may delay distension.

It is difficult to conceive any reason for this retardation of regeneration in the organic relations of parts of the oblique piece. Why should there be any difference between a portion of an oblique disc and a transverse disc at the same level of the body? Yet tentacle-regeneration on the former requires about twice as much time as on the latter.

The only plausible reason for this difference that has occurred to me thus far is the difference in the relation between the inrolled margin and the circulatory currents in the oblique and transverse pieces. It is evident, I think, that as soon as the oblique piece becomes closed by new tissue, and distension and stretching of the body-wall occurs, the chief tension upon the inrolled margins will coincide in direction with the plane of the disc. Thus on the lowest portion of the oblique disc the direction of tension will be obliquely upward and on the highest portion it will be obliquely downward. The result is that at the lowest portion of the margin the disc forms an obtuse angle with the body-wall, while on the opposite side the angle is acute; between these points the angle varies between these limits. Examination of the specimens shows this condition very clearly. At the uppermost portion of the disc the body-wall bends over sharply, almost as if actually folded, while the lower margin of the disc is rounded (Figs. 3 and 4) and the angle is much less.

It is evident that circulatory currents passing orally along the inner surface of the body-wall will exert much less pressure on the lower side than on the upper side, since the obtuse angle between disc and body-wall at the lower margin deflects them and permits escape toward the central portions of the body, while at the upper portion of the margin the currents strike an acute angle. On intermediate portions of the disc the angle, and consequently the pressure exerted, vary with the position from the maximum at the upper margins to the minimum at the lower margin. It is clear, moreover, that the angle between body-wall and disc at the lowest part of the margin of the oblique disc is greater than that between the body-wall and a transverse disc, while at the upper margin it is less. Half way between the upper and lower oblique margins it is the same as in the transverse disc.

If there is any relation between the circulatory currents and tentacle-regeneration we might expect acceleration of regeneration at the upper margin and retardation at the lower margin of the oblique disc. The retardation of regeneration is very evident, but as regards acceleration the data are insufficient to permit definite conclusions, though there are indications of a slight acceleration. In any case we could not expect the acceleration to be as great as the retardation since in ordinary cases of regeneration the inrolled margin forms a more or less acute angle with the body-wall and a difference in the size of this angle would make little difference in the pressure so long as the angle remains acute. As soon as the angle becomes obtuse however, the pressure must be reduced and as the angle increases the pressure becomes less.

In this, as in other cases, the regeneration of the labial tentacles proceeds in the same manner and sequence as that of the marginal tentacles, though much more slowly. As has already been mentioned, however, I do not know whether there is any localization of pressure in the regions where these tentacles appear.

My suggestions regarding the possible rôle of local pressure in bringing about the characteristic result in oblique pieces should be considered merely as an attempt to indicate how differences of local pressure may be effective here. The hypothesis must be applied to as many cases as possible before definite conclusions are reached. For the labial tentacles my data concerning local pressure are incomplete, as was pointed out in a previous paper ('04*b*). At present, however, I do not see any other probable explanation of the delay in the regeneration of the marginal tentacles on the lower portion of an oblique disc than that suggested above.

The gradual equalization in the length of the tentacles and the gradual reduction in the obliquity of the disc which are always features of the later history of oblique pieces, lead toward the establishment of symmetry and the typical form. Is there any satisfactory interpretation of these changes or must they be regarded for the present as inexplicable? They resemble in character certain of the phenomena which have led Driesch to assume the existence of an autonomistic principle or entelechy governing form. But I am convinced that an interpretation of an entirely different sort is possible.

In the first place it is necessary to recognize that the equalization of the tentacles and the reduction in obliquity of the disc are two wholly distinct phenomena due to wholly different factors. The equalization in length of the tentacles is the result of the same factors which cause the regeneration of tentacles of a certain length in typical transverse pieces and which bring about approximate equality in the length of tentacles in normal animals. If internal pressure plays a rôle in the latter cases, undoubtedly it is equally important in the former. Considering the oblique pieces, it is clear that as soon as the tentacles on the lowest part of the margin begin to grow conditions for their further growth are similar, as regards internal pressure and circulatory currents, to the conditions on the opposite margin. Each tentacle-bud forms a blind sac whose walls are growing tissue. Conditions of internal pressure in this sac do not differ on different parts of the margin, whatever the angle between disc and body-axis. The appearance of the tentacle-buds is delayed upon the lowest part of the margins and the possible cause for this delay has been indicated above.

If the length of the tentacles is dependent upon internal pressure, either general or local, there is no reason why the tentacles on the lowest part of the margin should not finally attain the same length as those on the highest part. They will attain this length later than the others because the first stages in their regeneration are delayed. Other factors such as the difference due to difference in level (see Child, '03*b*) may prevent the complete equalization in length, but there is always a close approximation to equality. Evidently then this regulative process does not differ essentially from others which have been discussed. The same principles which were applied to those are applicable here. In fine, the equalization in length of the tentacles is exactly what might be expected if internal pressure is the chief factor in determining their length.

The equalization of the tentacles presupposes nearly equal growth in the tentacular region on all parts of the circumference but the reduction in obliquity of the disc is apparently a compensatory process. Either the body-wall beneath the lower portion of the disc must undergo increase in length, or the body-wall beneath the highest portion must undergo decrease in length, or finally, both these changes must occur in order that the disc may attain a position at right angles to the longitudinal axis. No consideration of the conditions of internal pressure will, in my opinion, afford any means of explaining this compensatory regulative change. On the other hand I think there is little doubt that it is the result of certain characteristic activities of the animal, viz., its orientation in space. Loeb ('91) called attention to the remarkable power of orientation in *Cerianthus* and showed that in whatever position it was placed the effort was made to bring the longitudinal axis or at least its oral portion into a vertical position, and that having once attained this position the animal remains quiet until other stimuli bring about movement. My own observations agree fully with Loeb's as regards this point. The ability of pieces lying on the flat bottom of aquaria to bend the body at right angles and so lift the oral portion into a vertical position is most striking and has formed a constant feature of my experiments. In pieces with oblique discs an interesting modification of this orienting reaction has been observed. The oral portion of the body was brought into a vertical position and furthermore the effort was made by means of unequal contraction of the muscles on the two sides of the body to bring the disc into a horizontal position at right angles to the longitudinal axis. During the earliest stages of regeneration the pieces never react normally but after they become closed and distended the reactions gradually appear. In the oblique pieces this reaction is already very distinct at the stage of Fig. 5. When the pieces are left undisturbed they orient themselves so that the disc appears nearly horizontal and the body below it nearly vertical. That the muscles of the two sides must be unequally contracted is evident. A sudden tactile stimulus or other irritation causes a marked change in the relation of parts. The specimens undergo sudden contraction and the obliquity of the disc increases greatly. The stronger the stimulus and consequently the more complete the contraction of the muscles the greater the obliquity of the disc in such cases. Left to themselves, the animals gradually extend, become filled with water again and resume the former position. The increase of the obliquity of the disc in the contracted condition results from the difference in the degree of contraction at different points of the circumference in the distended oriented condition. A brief consideration will be sufficient to render this clear; in the normal animal a stimulus of this kind causes an equal degree of contraction in all the longitudinal muscles and there is no reason to suppose that the effect is different in the oblique pieces. The increase in the obliquity of the disc which accompanies contraction in oblique pieces must result from a difference in condition of the muscles on the opposite sides of the body. If for instance we suppose that the muscles of the body beneath the highest part of the oblique disc are partially contracted in the effort to bring the disc into a horizontal position it is evident that these muscles will contract less in consequence of the sudden stimulus than those on the opposite side of the body and consequently the angle between disc and longitudinal axis must decrease.

During the earlier stages of regulation the animal is unable to bring the disc into a completely horizontal position: it always remains somewhat oblique. Contraction at this stage causes the angle between disc and axis to approach its original value, *i. e.*, the disc becomes nearly as oblique as the original cut surface. As time goes on, however, the oriented animal brings the disc more and more nearly into the horizontal position and the obliquity of the disc in the contracted condition gradually decreases. In the later stages the obliquity is visible only in the contracted condition, the disc being apparently at right angles to the longitudinal axis in the distended oriented specimen.

This relation between the distended, oriented condition and the contracted condition is characteristic not only for pieces with oblique discs but for various other forms artificially produced. Whenever the artificial form interferes with the orientation a gradual compensatory regulation may be observed.

The most obvious conclusion and, I think, the correct one, regarding the regulation of the oblique discs is that the changes in the body-wall of the two sides of the body occur in consequence of the characteristic functional condition of the muscles. The muscles beneath the upper side of the disc being continually more or less contracted undergo a "functional" change in structure and lose a part of their original power of extension, while the muscles beneath the lower edge of the disc, being continually highly extended lose to some extent their original power of contraction. Or, considered from another point of view, there is functional atrophy on one side and functional hypertrophy on the other. The ectoderm and the entoderm of the body-wall follow these changes by similar growth or reduction. With each change further change becomes possible until the animal is finally able to orient itself in the typical manner, or in other words has attained the typical form. The most important point in this interpretation is the rôle played by the efforts of the animal to carry on its typical activities. In my opinion these efforts are the cause of the regulation. The animal attempts to perform certain acts and certain resulting conditions of the tissues give rise to a certain form. The reaction or attempt at reaction produces the form incidentally. The typical form is then in this sense the result, not the cause of the typical reaction.

No attempt at general application of this conclusion to morphology need be made at present, though the field for speculation is most attractive. I desire merely to call attention to the fact that this regulative process belongs in the same category with the changes in form of pieces of *Stenostoma* in consequence of the attempts of these pieces to carry on their typical movements (Child, '02, '03). In both cases the form is, speaking broadly, the result, not the cause of the behavior.

The Regeneration of Pieces from the Œsophageal Region.

Pieces with the oral cut surface at some level of the œsophageal region of the parent specimen and the aboral end at a greater or less distance below the œsophagus differ from pieces cut wholly aboral to the œsophagus only as regards rapidity of regeneration. In such pieces, containing a part of the œsophagus, the cut oral end of this organ unites with the cut body-wall and thus a functional mouth is formed within a day or two after section. Consequently such pieces become fully distended earlier and the tentacles appear somewhat sooner than in pieces in which a new mouth is regenerated.

In pieces representing only the œsophageal region or part of it (Fig. 7) the result usually differs widely from the preceding. In such pieces the cut ends of the œsophagus usually unite both orally and aborally with the cut body-wall and the œsophagus forms a tube extending completely through the piece and open at both ends to the exterior. Fig. 8 represents a longitudinal section of such a piece: in order that the œsophagus and body-wall may be readily distinguished in the diagrams the thin muscular layer of the former is not indicated. In these pieces there is no connection between the œsophagus and the enteron; water passing into the oral end of the œsophagus simply passes out again at its aboral end. Each intermesenterial chamber is thus shut off from all communication with the exterior and with adjacent chambers.

As regards tentacle-regeneration these pieces follow the usual course during the first few days, becoming more or less distended with fluid, which is secreted into the enteric cavity or diffuses through the body-wall. The body-wall becomes thinner at the oral end (Fig. 9) and tentacle-buds appear (Fig. 10), but these usually do not develop beyond the stage shown in Fig. 10. Occasionally they reach a length of 2–3 mm., but further than this their development never proceeds. The distension gradually decreases again after a week or two and the tentacles which had attained a length of 2–3 mm. decrease in size to mere buds. In cases where the tentacles never develop beyond the stage represented in Fig. 10 they are scarcely visible at all after two or three weeks.

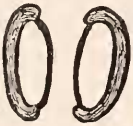
Fig. 8.


Fig. 9.

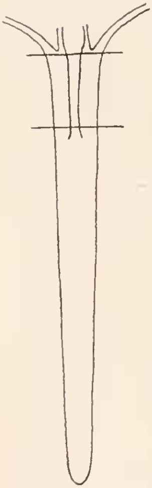
Fig. 7.


Fig. 10.


Fig. 11.


Fig. 12.

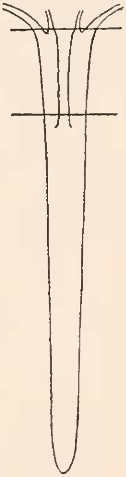
Fig. 15.


Fig. 13.


Fig. 14.

In some pieces, however, closure of the aboral end occurs sooner or later. This result may be attained in either of two ways· In one case the aboral cut surfaces of the œsophagus and body-wall fail to come into contact (Fig. 11) and the aboral end closes in the usual manner by union of the cut surfaces of the body-wall leaving the aboral end of the œsophagus free in the enteron (Fig. 14). Such pieces do not differ essentially from pieces in which the aboral end lies below the œsophageal region. They become distended in the usual manner and regenerate typically, but more rapidly than pieces in which regeneration of mouth and œsophagus occurs.

In the other case the cut surfaces of body-wall and œsophagus unite in the manner described above (Fig. 8), either completely or on a part of the circumference. Then it may happen that different portions of this region of growing tissue are brought into contact as in Fig. 12. The result is the union of all these parts and so the closure of the aboral end (Fig. 13). The connection between the œsophagus and body-wall is soon broken as the piece becomes more fully distended and the condition represented in Fig. 14 is attained. From this stage on regeneration at both ends proceeds in the typical manner. In some cases this closure of the aboral end and loss of connection between œsophagus and body-wall does not occur at first, but later the margins of the body-wall happen to come into contact perhaps in consequence of the gradual decrease in distension mentioned above as following the first increase, and thus closure occurs and is followed by renewed distension and rapid regeneration. Apparently the new tissue retains the power of making new unions for a considerable time after the cut surfaces of body-wall and œsophagus appear to be firmly united.

The final result in all œsophageal pieces in which the aboral end succeeds in closing aboral to the œsophagus is typical regulation with well-developed tentacles at the oral end and several millimeters of new tissue at the aboral end.

The data of a few experiments will serve to indicate the uniformity of results. In all cases *C. solitarius* was used.

Series 30.

September 28, 1902. — Seven œsophageal pieces were prepared from large specimens in good condition as follows: at the oral end of each animal a transverse cut was made through the disc at such a level that the marginal tentacles and margins of the disc were removed and the labial tentacles were cut off near their bases leaving stumps 1 mm. or less in length. Then a second cut was made through the œsophageal region near its aboral end. In the distal pieces thus formed the œsophagus extends from end to end of the piece, cut surfaces being present at each end (Fig. 15).

October 1. — 3 days after section. In all cases the cut margins of œsophagus and body-wall have united both orally and aborally in the same manner as in Fig. 8. All pieces are more or less distended and the oral margins slightly crenated in correspondence with the intermesenterial chambers.

October 4. — 6 days after section. All pieces with marginal tentacles, varying in length in different pieces from mere buds just visible to 2 mm.: labial tentacles still short stumps.

October 7. — 9 days after section. In two pieces the aboral end has closed as in Figures 12–14: these pieces fully distended; one with marginal tentacles 5 mm.; labial tentacles about 2 mm.; the other with marginal tentacles 3 mm., labial tentacles 1–1.5 mm.

In the five remaining pieces no aboral closure has occurred; the distension has decreased; the marginal tentacles have increased in length, though very slightly, since the previous examination, varying from minute buds to 1–2 mm.

October 15. — 17 days after section. One of the pieces which closed aborally with marginal tentacles 8–10 mm., labial tentacles 5–6 mm. At aboral end new tissue 1–2 mm.

The other closed piece was lost.

In none of the five remaining pieces has tentacle-regeneration proceeded since the previous examination. All appear almost completely collapsed and the tentacles are reduced to mere buds in all, being scarcely visible in some. One piece has begun to break up.

During the following two weeks the five pieces which did not close broke up into small pieces and died, a frequent occurrence in small pieces in which closure and regeneration do not occur. The one closed piece remained in good condition.

The difference between the piece that closed and the other five is striking. None of these five pieces ever regenerated marginal tentacles more than 1–2 mm. in length, and practically no regeneration of labial tentacles occurred. Yet the one piece which closed while no longer than these and differing from them only in that the œsophagus communicated with the enteron, regenerated marginal tentacles 8–10 mm. in length and labial tentacles 5–6 mm. and produced new tissue at the aboral end.

Series 32.

October 1, 1902. — Seventeen œsophageal pieces were prepared (Fig. 7) the distal cut being in this case slightly more aboral than in Series 30 (Fig. 15) so that both marginal and labial tentacles and most of the surface of the disc were removed.

During two weeks these were examined several times. In all pieces the cut margins of œsophagus and body-wall united both orally and aborally and in none did the closure of the body-wall across the aboral end occur. All of the pieces became slightly distended during the first few days and marginal tentacles appeared as minute buds less than 1 mm. in length. In no case did regeneration proceed beyond this stage: labial tentacles never appeared. At the end of two weeks some pieces were beginning to break up.

Series 47.

November 7, 1902. — Nine œsophageal pieces were prepared in the same manner as those of Series 32.

November 10. — 3 days after section. Still more or less completely collapsed: no tentacles visible on any.

November 12. — 5 days after section. One piece closed aborally, and well-filled with water; marginal tentacle-buds 0.5–1 mm. In the remaining eight pieces œsophagus and body-wall have apparently united both orally and aborally: these pieces are only slightly distended and show a slight crenation of the oral margin corresponding to the intermesenterial chambers.

November 20. — 13 days after section. During the interval since the last examination seven pieces closed aborally across the end of the œsophagus. These are fully distended and bear marginal tentacles 2–3 mm.; labial tentacles just appearing.

In two pieces the œsophagus remained open aborally. These are not fully distended and the marginal tentacles are minute buds about 0.5 mm.; labial tentacles are absent.

December 2. — 23 days after section. In the seven closed pieces regeneration has proceeded in the typical manner; marginal tentacles 5–6 mm.; labial tentacles 2–3 mm.

In the other two pieces regeneration has proceeded no further. They are still only slightly distended and with minute tentacle-buds.

In this series a larger proportion of the pieces closed aborally across the end of the œsophagus than in any other series of this kind. The date of this series was later in the year than that of any other, *i. e.*, the temperature of the water was lower and the distension of the pieces occurred much more slowly (see Child, '03*b*). It is probable that different parts of the aboral cut surfaces of the body-wall remained in contact for a longer time than in the other cases where distension occurred more rapidly, and that this prolonged contact made union possible in a greater number of cases than in other series. Other conditions such as difference in the degree of contraction of body-wall and œsophagus at the time the lower cut was made may have aided in bringing about this difference. However that may be, the point of chief importance, viz., the difference in regeneration between those pieces which did close aborally across the œsophagus and those which did not is as clear in this series as in others.

While the general result of these experiments is sufficiently clear special attention may be called to certain points.

In all cases in which the œsophagus remains open aborally and consequently does not communicate with the enteron the pieces never become fully distended and regeneration is slight. Pieces cut below the œsophageal region become well distended and the marginal tentacles may attain a length of 2–3 mm. before the mouth is formed. In my first paper ('03*a*) diffusion of water through the body-wall in consequence of the presence of soluble products of metabolism or other substances in the enteron was suggested as a possible cause of this first distension. It is also possible that secretion of fluid into the enteron occurs. In the œsophageal region the entodermal layer is thinner and undoubtedly of less functional importance than in the region aboral to the œsophagus, and it is reasonable to suppose that the accumulation of fluid in the œsophageal pieces would be much less rapid than in pieces from regions aboral to it. If this be admitted it follows that less water would enter these pieces and less distension would occur than in pieces aboral to the œsophagus. If the internal pressure affects tentacle-regeneration, less regeneration would occur in consequence of this slight distension in œsophageal pieces than in others where distension is greater, and this is actually the case.

It was noted that the pieces in which the œsophagus remains open aborally gradually collapse again in the course of a week or two. This collapse is probably due to a slow loss of the fluid which first caused the distension. If the body-wall is slightly permeable for substances in solution in the enteron the distension produced at first will gradually diminish since in consequence of continued starvation the amount produced gradually decreases. Whatever the exact nature of the process may be, a decrease in the distension occurs. As this process continues the regenerating tentacles also decrease in size instead of continuing to grow. This fact is important as showing the apparent close relation between tentacle-regeneration and internal water-pressure. In pieces cut below the œsophagus the regeneration of the mouth and the entrance of water through it serves not only to prevent decrease in the internal pressure but to increase it, and further growth of the tentacles takes place.

The most striking feature of the experiments is the difference in behavior as regards regeneration between the pieces in which the body-wall closes aborally across the œsophagus and those in which the œsophagus remains open aborally to the exterior. The pieces of the first kind begin to regenerate typically as soon as the closure occurs, while the others never produce anything more than marginal tentacles 1–2 mm. in length. In the introductory description of these experiments it was shown that in case of closure the connection between the aboral end of the body-wall and the œsophagus is severed and the œsophagus opens into the enteron (Figs. 12–14). The breaking of this connection, *i. e.*, the change from the condition shown in Fig. 13 to that of Fig. 14 is probably itself due at least in part to internal water-pressure, though the manner of its occurrence cannot be observed. Other factors, such as a difference in rapidity of growth or a difference in resistance between the tissue of the œsophagus and that of the body-wall may also play a part in the result. Whatever be the cause, the result is free communication between a fully formed œsophagus and the small enteric cavity with its intermesenterial divisions. If entrance of water through the œsophagus serves to maintain internal pressure in *Cerianthus* it is clear that in these pieces the pressure should at once become about equal to the pressure in a normal animal since the mouth opening and œsophagus are not regenerated structures but parts of the parent body and of full size. If internal water pressure affects regeneration we might expect in these pieces rapid regeneration, even more rapid than in pieces with regenerating mouth and œsophagus. Close comparison of these pieces with others in which mouth and œsophagus are formed by regeneration is difficult, since in the œsophageal pieces regeneration may be at first delayed until the aboral closure is complete, but the piece in Series 30 in which closure of the aboral end occurred between October 4 and October 7 and by October 15 the marginal tentacles were 8–10 mm. and the labial tentacles 5–6 mm., show the rapidity of regeneration. In fact regeneration in this piece after closure is more rapid than in any other case noted in my records with the exception of other pieces of the same kind.

The striking difference between œsophageal pieces in which the aboral closure occurs and those in which it does not constitutes evidence of great importance for the influence of internal water-pressure on regeneration. As regards the influence of local pressure due to circulatory currents in determining the position of tentacles and in their regeneration these experiments afford no direct evidence. They show merely that the greater the distension of the piece and consequently the more widely open the intermesenterial chambers and the greater the force and volume of the circulatory currents the more rapid is regeneration.

SUMMARY.

1. In pieces with oblique oral end the rapidity of tentacle regeneration differs on different parts of the disc, being greatest on the uppermost (most oral) portion and least on the lowest (most aboral) portion. The delay in regeneration on the lowest portion

as compared with the highest portion is much greater than the difference in rapidity of regeneration due to difference in level.

2. The only reason apparent for the difference in rapidity of regeneration on different parts of oblique discs is the difference in the angle between disc and body-wall, which is acute on the upper side of the disc while on the lower side it is obtuse. In consequence of this difference the local pressure exerted by the circulatory currents passing orally in the intermesenterial chambers must be much greater on the upper side of the disc than on the lower. If regeneration is influenced by these currents we should expect delay on the lower side, and it occurs in all cases.

3. The later equalization in length of the tentacles in pieces with oblique discs is due to the conditions which bring about equality in length of tentacles in normal animals. After the appearance of the tentacles the conditions as regards internal pressure are essentially similar on all parts of the oblique margin and equalization must be expected if the length of the tentacles is determined by internal pressure.

4. The reduction in the obliquity of the disc in oblique pieces is a compensatory process resulting from the attempt of the animal to orient itself with longitudinal axis vertical and disc horizontal. In the attempt at orientation unequal contraction of the muscles on different parts of the circumference occurs, and its continuation brings about changes in the tissues which lead gradually toward the establishment of the typical form. The form is the result, not the cause of the reaction.

5. In pieces cut wholly within the œsophageal region the two cut ends of the œsophagus usually unite with the cut ends of the body-wall, thus leaving the œsophagus open to the exterior at both ends. This method of closure isolates each intermesenterial chamber completely and prevents any direct communication between the enteron and the exterior.

6. Such œsophageal pieces become slightly distended at first in consequence of diffusion of water through the walls, but since the œsophagus is not in communication with the enteron the internal pressure remains far below that of the normal animal. Regeneration of the marginal tentacles begins in such pieces, but never proceeds beyond the formation of mere buds.

7. Occasionally an œsophageal piece closes aborally by union of the body-wall across the end of the œsophagus. In these cases communication between the œsophagus and enteron is established, the piece becomes fully distended, and regeneration proceeds in the typical manner.

8. The difference in regeneration between the "closed" and "open" œsophageal pieces is undoubtedly due to the difference in the degree of internal water-pressure.

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